Iranian Journal of Veterinary Science and Technology
Vol. 6, No. 1, 2014, 64-70

IJVST

Case report: Follow-up, diagnosis, clinical evidence, laboratory evaluation, and treatment of Idiopathic thrombocytopenia using human Intravenous Immunglobulin in a terrier dog

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Received: May 25, 2013 Accepted: September 17,2014

Abstract

The aim of the present case report is to describe the outcome, hematological and biochemical changes of treatment of idiopathic thrombocytopenia in a *Terrier* dog using *human intravenous immunglobulin*. The complete blood count, serum biochemistry, indirect immunofluorescence antibody and direct coomb's tests, radiography and sonography were performed. Laboratory findings indicated sever thrombocytopenia ($<15\times10^3/\mu l$) and mild leukocytosis. Increase in the erythrocyte sedimentation rate and total protein concentration were last up to 19 and 14 days, respectively .After it, areduction was observed and no relapse was reported. This case report provides the first successful treatment and clinical evidence of a terrier dog with idiopathic thrombocytopenia in which no underlying etiology has been identified. It was concluded that human intravenous immunoglobulin infusion (1 g/kg) is a safe treatment with a significant increase in platelet count without increase in hospitalization and the cost of patient care.

Keywords: dog, idiopathic thrombocytopenia, treatment, *human intravenous immunoglobulin*

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Introduction

Idiopathic thrombocytopenia (ITP) is classified as primary (Idiopathic) in which there is no apparent inciting cause of producing anti-platelet antibodies, or secondary to a known antigenic stimulus (Waner *et al*., 2000, Bexfield *et al*., 2005). This disease is a relatively uncommon hemostatic disorder, occurring in approximately 5% of thrombocytopenic dogs referred to veterinary institutions (Botsch *et al*., 2009). In human medicine, *human intravenous immunoglobulin (hIVIG)* is an important immunomodulatory treatment for various disorders; however, the epidemiology of ITP is not well-characterized in the general population (Kurata *et al*., 2011). In veterinary medicine, a few immune-mediated diseases have been examined for their response to *hIVIG* therapy, though no approved indications have been reported. Nevertheless, its use may be indicated in a wide variety of severe unresponsive immune-mediated diseases. The exact mechanism by which *hIVIG* modulates the immune system is not fully understood, although there is evidence to support several hypotheses (Lemieux *et al*., 2005). This case report aims to describe the outcome, hemobiochemical changes, and clinical evidence of treatment of ITP in a terrier dog with *hIVIG*.

Case presentation

A 8-year-old female *Terrier*, weighing 10.8 kg, with a history of vaccination, vomiting, lethargy and antifungal therapy with Griseofulvin was presented to Tehran University Veterinary Teaching Hospital. On physical examination, the dog was obese, with pale mucous membranes and melena. Other abnormalities such as buccal hemorrhage and lymphadenopathy were detected. This dog had an unhealthy appetite and diarrhea throughout following-up course.

Antibodies against *Ehrlichia canis (E. canis)* were determined by using fiuoehrlichia indirect immunofluorescence antibody (IFA) kit (FLUOEHRLICHIA, MegaCor, Austria). To perform direct coomb’s test (DCT), successful production of rabbit anti-canine immunoglobulin was carried out as reported previously (Hay and Westwood, 2002). Total (RBC) Red Blod Cell, total white blood cell (WBC) count, hemoglobin (Hb) concentration, packed cell volume (PCV) and RBC indices (i.e., MCV, MCH, MCHC) were determined with automatic hematological analyzer (Vet Hema-screen 18, Hospitex Diagnostics, Italy). Total protein (Tp) concentrations were determined using an automated analyzer (EppendorfEPOS Analyzer 5060, Germany) with standard biuret method. Erythrocyte sedimentation rate (ESR), Leukocyte differential counts, RBC morphological features, reticulocyte and platelet counts were evaluated using standard techniques.

Results and Discussion

An evaluation of peripheral blood smears indicated no evidence of spherocytosis. The dog had no positive DCT results. The results of thoracic and abdominal radiographies were normal. IFA testing was also negative for E. canis. Lymphadenopathy was observed. Conditions associated with ITP which were in the history of this dog are listed here; vaccination against canine distemper, hepatitis, leptospirosis, parainfluenza and parvovirus (DHLPP), immune-mediated anemia (IMHA), drug therapy with *Griseofulvin* and *Penicillin G* and ovariohysterectomy. The dog had a moderately regenerative anemia on presentation with a mild leukocytosis, consisting of a neutrophilia, monocytosis, and regenerative left shift, at the time of presentation. The reticulocyte count was tended to be highest on presentation and the platelet count often continued to drop for a variable period of time thereafter. Hematological values were recorded on day 1 (the day of admission) when a low point for platelet count were reached (Table 1). Results

obtained on the 1'st, 7'th and 19'th days of *hIVIG* treatment revealed a significant increase in the ESR levels and Tp concentration lasting up to 19'th and 14'th days following treatment (Table 2).

Table 1. Hematological findings in the dog with ITP on presentation.

Disease / Parameters	PCV %	Reticulocytes $\times 10^3/\mu l$	WBC $\times 10^3/\mu l$	Bands $\times 10^3/\mu l$	Neutrophils $\times 10^3/\mu l$	Monocytes $\times 10^3/\mu l$	Platelets $\times 10^3/\mu l$
ITP	38	>60	15	1.2	9.9	1.8	15

ITP idiopathic thrombocytopenia, PCV packed cell volume, WBC white blood cell

Table 2. ESR a and Tp b values on the 1'st, 7'th, 14'th and 21 days following *hIVIG* c treatment.

Days / Parameters	ESR (mm)	Tp (g/dl)
1'st	9	5.2
7'th	16	5.5
14'th	18	7.8
19'th	18	7.5
21'th	8	6.4

aESR erythrocyte sedimentation rate, b Tp total protein,c *hIVIG* human intravenous immunglobulin

The criteria for diagnosing ITP in the present case were based on thrombocytopenia, history, physical finding, no leukemia, exclusion of other identifiable causes of thrombocytopenia and response to treatment. In one study, the most common clinical signs of dogs with ITP at the time of presentation were petechiae (73%) and ecchymotic hemorrhages (56%) (Huang *et al*., 2012). Other reported signs included melena, oral bleeding, hematoma formation or excessive bleeding from wounds and venipuncture sites, pale mucous membranes, ocular bleeding, hematochezia, heart murmur, hematemesis, hematuria, vestibular signs, and hemoptysis (Huang *et al*., 2012, O'Marra *et al*., 2011, Smedile *et al*., 1997). Although the presence of melena in one study suggested a poor prognosis for affected dogs (O'Marra *et al*., 2011), it was rejected in the present case.

The present case had a recent IMHA and a history of exposure to drugs such as *Griseofulvin* and *Penicillin G*. In a controlled retrospective study, the recently vaccinated dogs with IMHA (vaccine IMHA group) had significantly lower platelet counts (P<0.05) compared to the unvaccinated IMHA group (Duval *et al*., 1996). The relationship between ITP in dogs and recent vaccination is controversial. Although a relationship has been documented in humans (Kurata *et al*., 2011; France *et al*., 2008), it has not been definitively established in dogs (Huang *et al*., 2012). The present case had been vaccinated against DHLPP two months before presentation. Since this dog had been complicated with IMHA previously, not being re-vaccinated during the study period, it is unknown whether vaccination would have triggered a recurrence of disease or not; however, the possibility of an association cannot be completely ruled out based on one case report and further investigations need to be done on the large sample populations. Although a history of drug administration is not necessary for incidence of ITP, dogs with a prior exposure known to be associated with this disease (Huang *et al*., 2012).

In dogs, leukocytosis with a left shift is common regarding to ITP (O'Marra *et al*., 2011, Bianco *et al*., 2009, Huang *et al*., 2012, Smedile *et al*., 1997). A mild leukocytosis, consisting of neutrophilia, monocytosis, and regenerative left shift was seen in the present case. In an inflammatory response, extravasation of leukocytes is followed in time first by increased release and then by increased production of leukocytes by the bone marrow. The left shift that precedes a leukocytosis reflects both the magnitude and acuteness of onset of the inflammatory response (Janeway *et al*., 2005).

A variety of diseases has been known to be associated with thrombocytopenia in dogs (Waner *et al*., 2000, Bexfield *et al*., 2005, Huang *et al*., 2012). Clinical and hematological abnormalities are often nonspecific during E. canis infections, and co-infections with other tick transmitted agents are common (Karlikaya *et al*., 2007, Scorpio *et al*., 2008). The present case was excluded from any currently or historically underlying illness and IFA testing for *E. canis* was negative. The DCT was applied for diagnosis of IMHA and no positive result was reported. Considering laboratory findings and DCT result, possibility of IMHA excluded from this dog.

Studied dog was spayed female with 8 years old. In one study on 48 dogs with ITP, the median age at the time of diagnosis was 7 years; 25 were spayed females, 18 were castrated males, 3 were males of unknown neuter status, 1 was an intact female and 1 was a female of unknown neuter status (Huang *et al*., 2012).

Despite the benefit obtained by many patients from vigorous treatment (corticosteroids, chemotherapeutic agents, splenectomy, blood transfusion), some do not respond and some suffer frequent, occasionally fatal relapses (Marion *et al*., 1985, O'Marra *et al*., 2011, Smedile *et al*., 1997, Huang *et al*., 2012). In a study carried out by Marion *et al*. (1985) of those dogs with ITP that treated with various combinations of immune-suppressive drugs, 79% responded initially from which 41% relapsed, later (Marion *et al*., 1985). Karlikaya *et al*. (2007) reported that *hIVIG* treatment may cause remarkable but clinically insignificant changes in the biochemical and hematological profiles of treated patients (Karlikaya *et al*., 2007). *HIVIG* has been used to treat canine IMT and doses from 0.5 to 1.5 g/kg may be effective (Scott-Moncrieff *et al*., 1997, Bianco *et al*., 2009). In former study, dogs with IMT had failed to respond after 11 days of prednisone therapy. Platelet count was 5000 prior to *hIVIG* infusion, and after the specific use of *hIVIG* counts increased to 145×10^3/µl by 24 hours, and were normal by day 4 (Scott-Moncrief *et al*., 1997). In the latter one, compared with corticosteroids alone, adjunctive emergency therapy of a single *hIVIG* infusion was associated with a significant reduction in platelet count recovery time (Bianco *et al*., 2009). In the present case, the dog did not respond to any type of corticosteroids therapy during 3 weeks of monitoring, till *hIVIG* (1 g/kg) was administered as an intravenous infusion. Significant increase in platelets numbers occurred within 72h. Counts increased to 55 $\times10^3$/µl within 16 hours, 120×10^3/µl by 72 hours. The response time to reach a platelet count to the reference interval (200-600×10^3/µl) was 13 days. The dog carefully monitored and no record of immediate or delayed adverse reactions was reported over a 4-month period. Rapidity of response offers tremendous benefits, including a decreased risk of hemorrhage, decreased need for multiple transfusions, and shortened hospital stay.

The increase in ESR is believed to be a consequence of enhanced rouleaux formation caused by the infused IgG (Lee *et al*., 2005). In one study, this finding has been confirmed by showing a strong positive correlation between the IgG and ESR level (Karlikaya *et al*., 2007).

Conclusions

In conclusion, the results of this case study indicated that *hIVIG* infusion following drug therapy with corticosteroids could be a novel promising therapy with the potential of short-term remission and sustained response and without any adverse effects or increasing hospitalization in a terrier dog suffered with ITP. It should be noted, this is not a prospective study but a case report in which the experiences of the use of *hIVIG* is being described in one terrier dog so results could not be necessarily over generalized for other breeds of dogs.

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پی گیری، تشخیص، شواهد درمانگاهی، ارزیابی آزمایشگاهی و درمان با استفاده از تزریق داخل وریدی ایمنوگلوبولین انسانی در یک قلاده سگ تریر مبتلا به ترومبوسیتوپنی نامعلوم

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دریافت مقاله : ۱۳۹۲/۰۳/۰۴ پذیرش نهایی: ۱۳۹۳/۰۶/۲۶

چکیده

در تحقیق حاضر تغییرات خونی – بیوشیمیایی و نیز نتایج درمانی با استفاده از تزریق ایمنوگلوبولین داخل رگی انسانی در یک قلاده سگ تریر مبتلا به ترومبوسیتوپنی با عامل ناشخص توضیح داده می شود. آزمایشات مختلف همچون شمارش کامل سلول های خونی، بیوشیمی خون، تست ایمنوفلورسانس غیرمستقیم، کومبس مستقیم، رادیوگرافی و سونوگرافی انجام گرفت. یافته های آزمایشگاهی کاهش شدید پلاکت ها (< ۱۵۰۰/µl) و لکوسیتوز ملایم را نشان دادند. افزایش قابل ملاحظه ای در مقادیر ضریب ته نشت گلبول های قرمز تا روز ۱۹ و پروتئین تام تا روز ۱۴ متعاقب درمان با ایمنوگلوبولین انسانی مشاهده شد و سپس این مقادیر کاهش یافته و عود مجدد بیماری گزارش نشد. این گزارش درمانگاهی اولین درمان موفقیت آمیز با ایمنوگلوبولین انسانی در یک قلاده سگ تریر مبتلا به ترومبوسیتوپنی نامعلوم با شواهد کلینیکی مشخص را نشان می دهد و اینکه این دارو با اثرات درمانی طولانی مدت می تواند یک شیوه درمانی موثری در گروه بزرگی از بیماری های با واسطه ایمنی همچون ترومبوسیتوپنی نامعلوم مورد استفاده قرار گیرد. نتیجه اینکه تزریق داخل رگی ایمنوگلوبولین انسانی با دوز (1 g/kg) در موارد ذکر شده، با افزایش معنی دار پلاکت ها و کاهش زمان بستری و مراقبت های ویژه در بیمارستان و هزینه های مربوطه، یک راهکار درمانی ایمن محسوب می شود.

واژگان کلیدی: سگ، ترومبوسیتوپنی ناشناخته، درمان، ایمنوگلوبولین داخل رگی انسانی